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THE FORMATION AND TAXONOMIC STATUS OF THE MICROBASIC Q-MASTIGOPHORE NEMATOCYST OF SEA ANEMONES

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Some 27 different types of nematocysts have been described to date from members of the Phylum Cnidaria (Mariscal, 1974c). One of the commonest types, the microbasic mastigophore nematocyst, was described by Weill (1934) as having a well defined shaft (enlarged basal portion of thread) less than three times the capsule length. Carlgren (1940) later subdivided the microbasic mastigophores into two distinct types, the microbasic b- and microbasic p-mastigophores, based on the structure of the shaft. The shaft of a discharged microbasic b-mastigophore tapers gradually into the thread, while that of a discharged microbasic p-mastigophore abruptly narrows down to form the thread (*e.g.*, see Fig. 4–14 of Mariscal, 1974c). The lower portion of the shaft of an undischarged microbasic p-mastigophore has a prominent inverted V-shaped notch, while the microbasic b-mastigophore lacks this feature.

Cutress (1955) has described a third type from sea anemone acontia, the microbasic q-mastigophore, characterized by the possession of a harpoon-like dart which is propelled out of the capsule during discharge. Iwanzoff (1896) was apparently the first to notice this phenomenon, and Weill (1934) has also described it in association with two different nematocysts: the microbasic mastigophore and microbasic amastigophore.

The amastigophore is similar to the microbasic mastigophore, but as originally described, lacked a terminal thread beyond the shaft (Weill, 1934). However, as Cutress (1955) has pointed out, a very short fragment of thread may be present, either on the discharged shaft or left behind attached to the inside of the discharged capsule wall.

Iwanzoff (1896), Weill (1934), Hand (1961) and Schmidt (1969) suggest that the dart represents spines from the shaft which have somehow fused together, or otherwise failed to separate, during discharge. These authors do not consider the presence of a dart to be sufficient grounds for the erection of a new category of nematocyst. Cutress (1955), on the other hand, has argued that the dart is an unattached, discrete structure which is consistently found in the capsules of certain nematocysts and characterizes an entirely new type of nematocyst. In order to resolve this controversy and because darts represent one of the most curious features yet described in association with nematocysts, dart-forming nematocysts were examined in the present study using both phase contrast and scanning electron microscopy. Specific problems addressed include the relative proportion of darts formed by tentacle nematocysts as opposed to acontial and column nematocysts, the type(s) of nematocysts capable of forming darts, the number of darts formed by nematocysts within a particular category, the method of formation of darts and their possible function in the lives of the organisms involved. Based on the information

obtained from a variety of sources in the present study, it will be seen that the microbasic q-mastigophore should not be considered as a valid taxonomic entity.

Materials and Methods

The sea anemone material examined in the present study included the tentacles and acontia of *Aiptasia pallida* (Verrill, 1864), the tentacles and acontia of *Bartholomea annulata* (Le Sueur, 1817) and the tentacles and column vesicles of *Bunodeopsis antilliensis* Duerden, 1897. *Aiptasia* was found on floating docks and *Bunodeopsis* on floating *Sargassum* in the vicinity of Alligator Harbor on the north Florida Gulf Coast. *Bartholomea* was collected from coral heads off Key Largo, Florida.

Fixation of tissues for scanning electron microscopy (SEM) was in Parducz's (1967) Fixative. The subsequent preparation for Freon critical point drying followed the procedures of Mariscal (1974a, b). The dried tissue was coated with gold-palladium and then examined in the Cambridge Stereoscan S4–10 scanning electron microscope.

Positive and negative (anoptral contrast) phase contrast microscopy was done on a Reichert Zetopan microscope with a Nikon AFM photomicrographic attachment.

Counts of nematocysts at 400 × magnification were made from three fields of view of two different preparations, each for a total of six counts per nematocyst type observed. Nematocyst terminology follows that given in Mariscal (1974c).

Results

New nematocyst types: microbasic amastigophore, types A and B; microbasic p-mastigophore, types A and B

As Weill (1934) points out, two different types of nematocysts may be involved in dart formation: the microbasic mastigophore (Fig. 1) and the microbasic amastigophore (Fig. 2). Of the former, only the microbasic p-mastigophore is thought to be capable of forming darts, and because of this, Cutress (1955) has erected a new category which he calls the microbasic q-mastigophore.

Both the microbasic p-mastigophore and microbasic amastigophore can be subdivided into two additional types, A and B, based on the size, shape, arrangement and relative abundance of spines on the everted shaft. Although these differences are apparent in phase contrast microscope observations (Fig. 2), SEM observations were required to determine their exact nature.

The microbasic amastigophore type A is characterized by a widely spaced and well-differentiated helical coiling of spines whose length is equal to, or less than, the diameter of the shaft (Fig. 3). The tips of these spines in our preparations are truncated rather than pointed, as they are in many nematocysts.

The shaft of the microbasic amastigophore type B, on the other hand, has many flat, blade-like spines which are as long or longer than the shaft diameter and which have pointed tips (Fig. 3). In addition, either due to spine length or number, their arrangement on the shaft is not obvious, although it is assumed to be a triple helix based on observations of other species (Mariscal, 1974c). Because of the size and shape of its spines, the discharged shaft of the microbasic amastigophore type B tends to have a bottle-brush appearance (Fig. 4).

The microbasic p-mastigophore may also be subdivided into types A and B, using the same criteria as in the case of the microbasic amastigophores. In both cases, only the type B nematocysts (possessing the long, blade-like spines) are capable of forming darts (Table I).

Relative frequency of dart formation from different regions of the anemone

Both the microbasic amastigophore type B and microbasic p-mastigophore type B nematocysts are capable of forming darts. However, depending on the location in the anemone of the type B nematocyst, there is a striking difference in the relative number of darts formed. Dart formation by either type of nematocyst is rare in the tentacles, but relatively common by the same nematocyst types located in the lower column vesicles or acontia as shown by Tables I and II. For example, only 1.1% or less of the type B amastigophores or mastigophores from the tentacles formed darts, while 27.4% and 28.6% of the mastigophores from the acontia formed darts (Table IIA). Regardless of whether the same nematocyst types (*e.g.,* Table IIA, *B. annulata*) or a different nematocyst type (*e.g.,* Table IIA, *A. pallida*) was involved, the nontentacle nematocysts were the only ones which commonly formed darts (*cf.,* Table I).

Mechanism of dart formation

Examination of acontia or column vesicles with the phase contrast microscope revealed darts of varying lengths lying next to microbasic amastigophores and microbasic p-mastigophores whose shafts were partially devoid of spines (Figs. 5, 6, 7). Occasionally the dart could be found poised on the tip of the everted shaft, reminiscent of the detachable head of a spear gun or harpoon (Fig. 8). In some cases, the dart could be observed at the tip of a partially everted shaft (Fig. 9). In such cases, it appeared that eversion of the shaft had been halted part way through discharge, perhaps due to the dart acting as a plug or somehow interfering with the eversion process. The conclusion from these light microscopical observations was that the dart appeared to be formed by a fusion or adhesion of the shaft spines during discharge. This hypothesis was confirmed by the use of scanning electron microscopy.

SEM observations of the tentacles of *Bartholomea annulata* containing microbasic p-mastigophore nematocysts halted part way through discharge clearly showed that the spines had been stripped off the everted shaft for varying distances (Fig. 10). The length of each of the darts in such cases was generally proportional to the length of the denuded portion of the shaft: the shafts bearing short darts had the spines missing for only a short portion of their length and *vice versa* (Figs. 10, 11). A closer examination of the tip of such a shaft clearly shows the contribution of the shaft spines to the formation of the dart, as well as suggesting that failure of some of the spines to sever their connection with the previously everted portion of the shaft may have been responsible for the incomplete eversion of this nematocyst (Fig. 12).

Interestingly enough, it is even possible to determine the point of origin on the shaft of some of the individual spines which form the dart. Note in Figure 13 that some spines incorporated into the dart are intact, having broken free

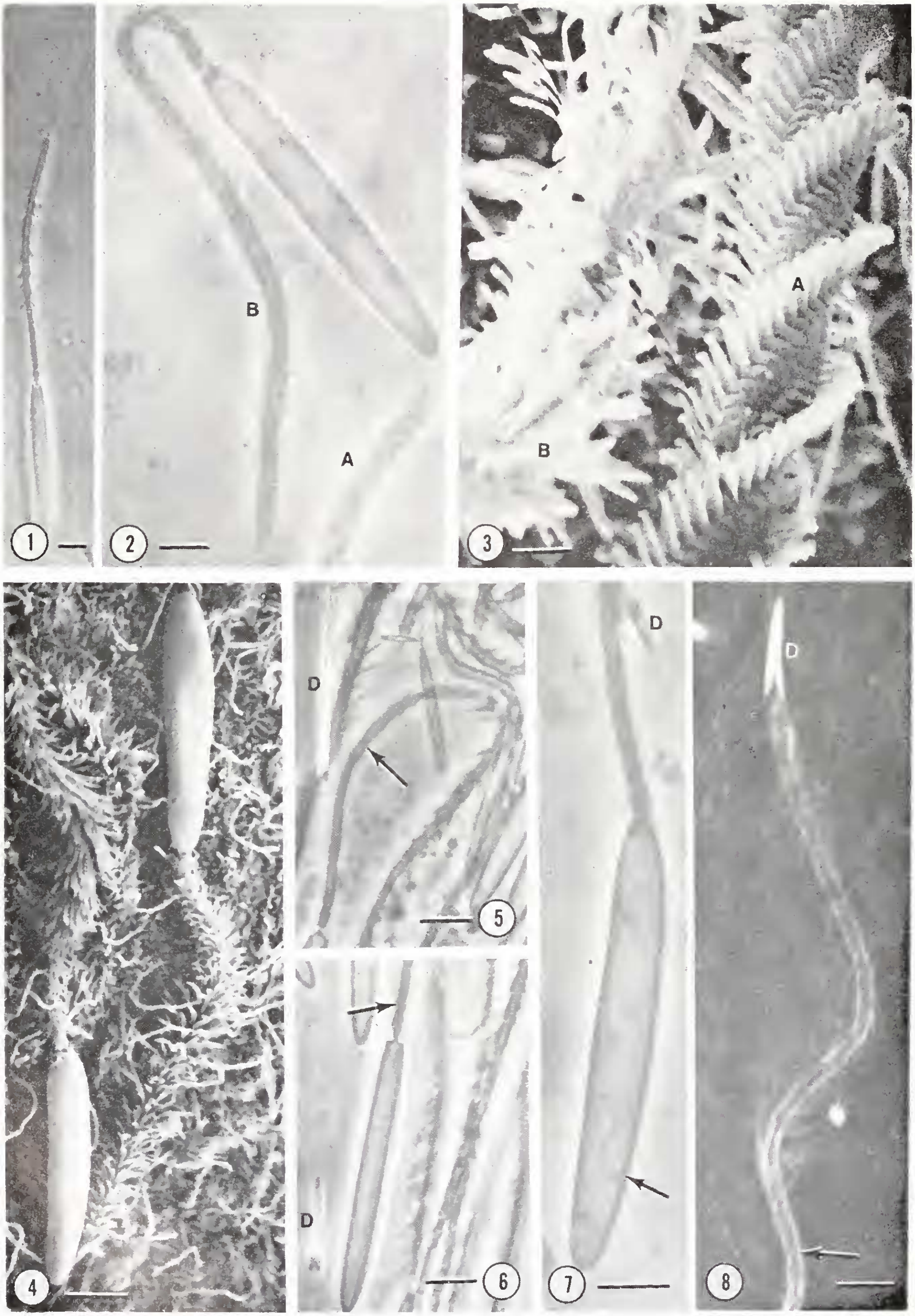
1
2
B
A
3
A
B
4
D
5
D
6
D
7
D
8

TABLE I

Tentacle, column and acontial nematocysts of the sea anemones Aiptasia pallida, Bartholomea annulata *and* Bunodeopsis antilliensis *showing the relative frequency of dart formation.*

Species of anemone and nematocyst type	Location in anemone	Relative frequency of dart formation
Aiptasia pallida		
MA, type B	Tentacles	rare
Mp-M, type B	Acontia	common
Bartholomea annulata		
Mp-M, type B	Tentacles	none
Mp-M, type B	Acontia	common
Bunodeopsis antilliensis		
MA, type A	Tentacles	none
MA, type B	Tentacles	rare
MA, type A	Lower column vesicles	none
MA, type B	Lower column vesicles	common

Key: MA = microbasic amastigophore; Mp-M = microbasic p-mastigophore.

at the point where the flanged base of the spine joins the shaft surface (*i.e.*, those spines making up the basal quarter of the dart length). Further along towards the tip of the same dart, it will be seen that the spines became broken during incorporation into the dart. Their stubby basal portions can still be seen adhering to the everted shaft (Figs. 11, 13).

As with length, the shape of the dart may also be variable. In some cases, the dart tapers directly into a sharp point (*e.g.*, Figs. 6, 13), while in other cases it has a slightly enlarged, spear-like tip (Figs. 5, 14).

Possible functions of the dart

In order to test the idea that darts could be propelled with sufficient force to penetrate another organism, free-swimming *Artemia* nauplii were allowed to con-

FIGURE 1. Phase contrast photomicrograph of microbasic p-mastigophore, type B, from acontium of *Aiptasia pallida*. Scale bar is 10 μm.

FIGURE 2. Phase contrast photomicrograph of microbasic amastigophores, types A and B, from column vesicle of *Bunodeopsis antilliensis*. Scale bar is 5 μm.

FIGURE 3. Scanning electron micrograph (SEM) of microbasic amastigophores, types A and B, from column vesicle of *Bunodeopsis antilliensis*. Scale bar is 1 μm.

FIGURE 4. SEM of microbasic amastigophores, type B, on tentacle of *Aiptasia pallida*. Scale bar is 5 μm.

FIGURE 5. Phase contrast photomicrograph of detached dart (D) lying next to shaft denuded of spines (arrow) of microbasic p-mastigophore, type B, from acontium of *Aiptasia pallida*. Scale bar is 10 μm.

FIGURE 6. Phase contrast photomicrograph of detached dart (D) lying next to capsule of microbasic p-mastigophore, type B, with shaft denuded of spines (arrow) from acontium of *Aiptasia pallida*. Scale bar is 5 μm.

FIGURE 7. Phase contrast photomicrograph of microbasic amastigophore, type B, from column vesicle of *Bunodeopsis antilliensis*. Note short thread (arrow) left attached to inner wall of capsule and the basal portion of shaft lacking spines below dart (D). Scale bar is 5 μm.

FIGURE 8. Negative phase contrast photomicrograph of everted shaft of microbasic amastigophore, type B, from column vesicle of *Bunodeopsis antilliensis*. Note basal portion of shaft lacking spines (arrow) and dart (D) poised on tip of shaft. Scale bar is 5 μm.

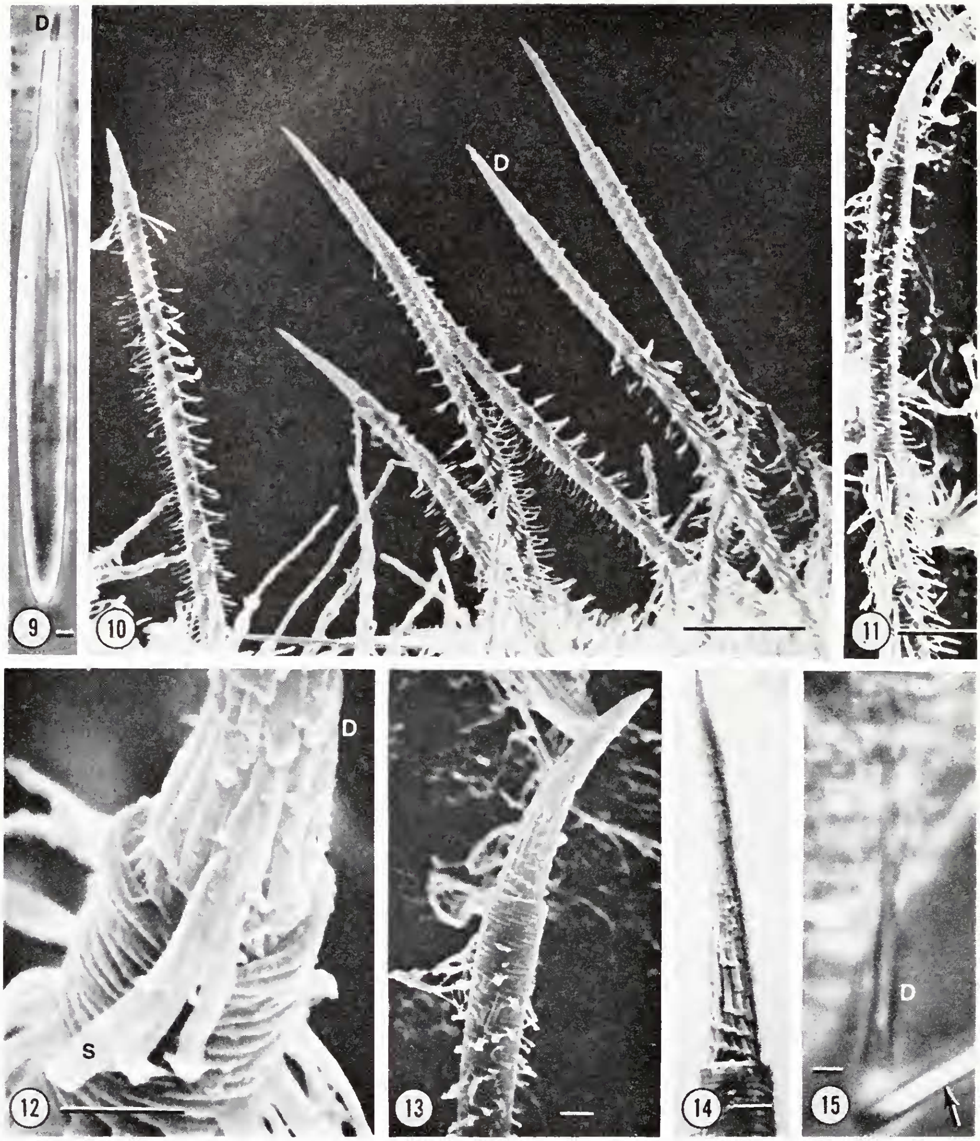

FIGURE 9. Phase contrast photomicrograph of microbasic amastigophore, type B, from tentacle of *Aiptasia pallida* with dart (D) at tip of partially everted shaft. Note that everted portion of shaft lacks spines. Scale bar is 1 μm.

FIGURE 10. SEM of microbasic p-mastigophores, type B, with darts (D) at tips of partially everted shafts on tentacle of *Bartholomea annulata*. Note everted shafts with missing or broken spines. Scale bar is 10 μm.

FIGURE 11. SEM of distal portion of microbasic p-mastigophore, type B, from tentacle of *Bartholomea annulata* showing dart and shaft with numerous missing or broken spines. Scale bar is 4 μm.

FIGURE 12. SEM of partially everted shaft of microbasic p-mastigophore, type B, from tentacle of *Bartholomea annulata* showing incorporation of shaft spines (S) into formation of dart (D). Scale bar is 1 μm.

TABLE II

Nematocysts from different species of sea anemones showing the actual percentage of dart formation under different conditions. Each type of nematocyst was counted at a magnification of 400× using three different fields of view from two preparations each for a total of six counts.

Species of anemone and nematocyst type	Location in anemone	Total number of counts at 400×	Total number of nematocysts	Total number of darts	Per cent darts observed
A. Nematocysts from excised tentacles and acontia					
Aiptasia pallida					
MA, type B	Tentacles	6	175	2	1.1
Mp-M, type B	Acontia	6	190	52	27.4
Bartholomea annulata					
Mp-M, type B	Tentacles	6	95	0	0
Mp-M, type B	Acontia	6	227	65	28.6
B. Nematocysts from *in situ* tentacles and acontia discharged on glass coverslips coated with *Artemia* nauplius extract.					
Bartholomea annulata					
Mp-M, type B	Tentacles	6	94	0	0
Mp-M, type B	Acontia	6	222	54	24.3

Key: MA = microbasic amastigophore; Mp-M = microbasic p-mastigophore.

tact the extruded acontia of *A. pallida* and their exoskeletons examined microscopically to determine if any darts might be present. It was determined that darts could penetrate the exoskeleton of a crustacean (Fig. 15).

It is also possible that some partially discharged darts projecting out at right angles from the tentacle surface could be effective in deterring a potential predator, especially if contact caused these nematocysts to complete eversion and discharge their contained darts (*e.g.,* see Fig. 10).

One might expect that if dart formation had a defensive function, food stimuli might not affect the relative numbers of darts formed. Therefore, the effect of food stimuli on dart formation was also tested in a living *B. annulata* anemone. Food stimulus-coated coverslips (with *Artemia* extract) presented to the intact tentacles and acontia of this anemone did not alter the relative proportion of darts formed by tentacle and acontial nematocysts; similar to the results shown in Tables I and IIA, no darts were formed by the tentacle microbasic p-mastigophores, while 24.3% of this nematocyst type in the acontia did form darts (Table IIB).

FIGURE 13. SEM of tip of partially everted shaft and dart of microbasic p-mastigophore, type B, from tentacle of *Bartholomea annulata.* Note whorls of intact spines forming basal portion of dart. Scale bar is 1 μm.

FIGURE 14. SEM of dart at tip of partially everted microbasic p-mastigophore, type B, on tentacle of *Bartholomea annulata.* Note flanged bases of intact spines at base of dart and enlarged tip of dart. Scale bar is 1 μm.

FIGURE 15. Phase contrast photomicrograph of dart (D) from microbasic p-mastigophore, type B, from acontium of *Aiptasia pallida* embedded in exoskeleton of *Artemia* nauplius larva at left. Note second dart (arrow) lying free in medium next to *Artemia.* Scale bar is 1 μm.

Discussion

As Cutress (1955) points out, and we have been able to verify, many amastigophores do in fact have a very short thread, all or a portion of which may be left behind attached to the inside of the capsule wall following discharge. Thus, at least some amastigophores could be considered to be microbasic mastigophores with very short threads. This has prompted several workers (Cutress, 1955; Schmidt, 1969, 1972, 1974) to propose the elimination of the category of amastigophore entirely. Based on our studies, we can generally sympathize with this point of view. For example, elimination of the amastigophore might make it easier to explain why both the amastigophore and microbasic p-mastigophore can form darts and why each have two sub-types, A and B.

On the other hand, we believe it would be premature at this point to eliminate the amastigophore completely until more nematocysts of this and other types (especially the microbasic p-mastigophores) from additional species can be examined in detail with the electron microscope. In addition, the tentative retention of the amastigophore can perhaps be justified on functional grounds, in that regardless of what it is called, it is at least apparently functioning as an amastigophore since its short thread may often be left behind in the capsule or broken during discharge.

Based on our observations, only the type B amastigophores and microbasic p-mastigophores are capable of forming darts. Their relatively greater density and tighter packing probably allow the longer and more delicate spines of this nematocyst to adhere together and to be stripped from the shaft to form the dart during eversion.

The formation of darts by the type B nematocysts from either the acontia or column vesicles is of interest because of the possible adaptive significance to the species involved. Acontia have generally been considered to have a defensive function because they are often extruded through the body wall when an anemone is disturbed and because they bear large numbers of penetrating nematocysts (Hyman, 1940; McLean and Mariscal, 1973). The fact that darts are commonly formed by nematocysts only from the acontia or column vesicles suggests that they may play some role in the life of the anemones involved, perhaps in defense from benthic predators such as nudibranchs. The latter often contact the column in the vicinity of the vesicles or cinclides (through which the acontia are extruded) during the initial stages of predation (Waters, 1973; Conklin and Mariscal, 1977). Waters (1973), for example, reports that first contact of the predatory nudibranch *Aeolidia* with the acontiate anemone *Metridium* initiated contraction of the anemone and caused its acontia to move toward the nudibranch. Further contact by the nudibranch caused the anemone to contract more strongly and to extrude its acontia through the body wall in the region of contact. Contact of an acontium with the nudibranch caused the latter to contract strongly, especially at the point of contact, and to move away from the acontium while secreting copious amounts of mucus.

Because dart formation was, on the average, some 27 times more frequent in acontial nematocysts than in tentacle nematocysts and because darts can penetrate other organisms, it is suggested that the formation of darts by an anemone may confer some selective advantage to the species. For example, a penetrating nematocyst which anchored prey organisms to an anemone's tentacles would obviously be

of importance in preventing prey from escaping before ingestion. However, one would not expect selection to favor the use of the same type of nematocyst against an organism which preyed on anemones. In this case, it would be to the advantage of the anemone simply to deter or drive away a potential predator, perhaps by the use of a detachable spear head or arrow which was *not* anchored to the anemone's tissues.

It is concluded that although the microbasic q-mastigophore does not appear to be a distinct morphological type (since the dart forms from the fused spines of other nematocysts during discharge), it may represent a functional type of nematocyst. Should this be the case, and should dart formation confer some selective advantage to a species, then perhaps we are observing the evolution of a new morphological type of nematocyst. Thus in the tentacles, where selection might favor nematocysts which are capable of injecting toxin and aiding in prey capture, there would be little selective pressure to form darts. On the other hand, in the acontia or column vesicles which might first be contacted by a potential benthic predator, we might expect to, and in fact do, find a much greater frequency of dart formation. An experimental study investigating the relative success among aeolid nudibranchs preying on anemones possessing dart-forming nematocysts and those lacking these cnidae would be of interest in this regard.

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Summary

Detachable darts, supposedly diagnostic of the microbasic q-mastigophore nematocyst, are formed by the adhesion of shaft spines of two different nematocysts: the microbasic amastigophore and microbasic p-mastigophore. Based on the morphology of their shaft spines, each of the nematocysts can be subdivided into two additional types, A and B. Only the type B nematocysts are capable of forming darts.

Dart formation by tentacle nematocysts was rare, but was relatively common among type B acontial or column vesicle nematocysts. Experiments showed that darts were formed by acontial nematocysts, for example, about 27 times more frequently than by tentacle nematocysts, both intra- and interspecifically. However, only a relatively small percentage of type B nematocysts appeared capable of forming darts in the material examined.

Darts were propelled out of the capsule with sufficient force to penetrate crustacean exoskeleton. Because of this and evidence from the experimental studies, it is suggested that dart formation may play a role in the defense of some anemones against benthic predators. Although possibly having functional significance for those anemones possessing the appropriate nematocyst, dart formation caused by the fusion of shaft spines among only a certain percentage of the nematocyst population indicates that the microbasic q-mastigophore is not a valid nematocyst type.

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